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(54) Title: TREATMENT OF GASTROINTESTINAL DISEASE WITH PPAR MODULATORS

(57) Abstract

Methods and compositions are provided for treating a host having a gastrointestinal (GI) disease by administering to the host a composition containing a pharmaceutically effective amount of a modulator of a Peroxisome Proliferator Activated Receptor (PPAR), particularly a modulator of PPARγ. Screening methods are also provided for identifying PPAR modulators which utilize cells derived from gastrointestinal tissue which express PPARs.

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TREATMENT OF GASTROINTESTINAL DISEASE WITH PPAR MODULATORS

Field of the Invention

This invention relates to methods and pharmaceutical compounds for treating gastrointestinal diseases. The invention also relates to screening methods for identifying useful pharmaceutical compounds for treating gastrointestinal diseases.

Background of the Invention

A. Peroxisome proliferator activated receptors (PPARs)

Peroxisome proliferator activated receptors (PPARs) constitute a subfamily of the nuclear hormone receptors. Three distinct PPARs, termed α, δ (also called β, NUC-1 or FAAR) and γ, each encoded by a separate gene and showing a distinct tissue distribution pattern, have been described [Reviews: Desvergne, B. and Wahli , W., *Birkhauser. 1*: 142-176 (1994); Green, S., *Mutation Res. 333:* 101-109 (1995); Schoonjans, K. *et al., Biochim. Biophys. Acta. 1302:* 93-109 (1996); Schoonjans, K. *et al., J. Lipid Res. 37:* 907-925 (1996)]. Although it was known that PPARs are activated by a wide variety of chemicals including fibrates, phthalates and fatty acids, PPARs were initially considered orphan receptors, since no direct binding of these compounds to the receptors could be demonstrated.

Activated PPARs heterodimerize with retinoid X receptors (RXRs), another subfamily of nuclear hormone receptors, and alter the transcription of target genes after binding to PPAR response elements (PPREs). A PPRE typically contains a direct repeat of the nuclear receptor hexameric DNA core recognition motif, an arrangement termed DR-1 when recognition motifs are spaced by 1 nucleotide [Schoonjans, K. *et al., J. Lipid Res. 37:* 907-925 (1996)].

Recently, ligands that induce the transcriptional activity of PPARα (fibrates and leukotriene B4) and γ (prostaglandin J derivatives and thiazolidinediones) have been identified [Devchand, P. R. *et al., Nature 384*: 39-43 (1996)].

Numerous PPAR target genes have been identified so far [Review: Schoonjans, K. *et al., Biochim. Biophys. Acta. 1302:* 93-109 (1996)], and additional target genes continue to be identified [Hertz, R. *et al., Biochem. J. 319*: 241-248 (1996); Ren, B. *et al., J. Biol.*

Chem. 271: 17167-17173 (1996)]. Since they are activated by various fatty acid metabolites as well as several drugs used in the treatment of metabolic disorders, PPARs can be considered as key messengers responsible for the translation of nutritional, pharmacological and metabolic stimuli into changes in gene expression.

I. PPARα

PPARα was the first PPAR to be identified [Isseman, I. and Green, S., *Nature 347*: 645-650 (1990)]. Among the known PPARs, PPARα is most effectively stimulated by peroxisome proliferators and mediates the response to peroxisome proliferators in rodents [Lee, S. S. T. *et al., Mol. Cell. Biol. 15:* 3012-3022 (1995)]. Recently, it was shown that peroxisome proliferators of the fibrate group are not only activators of PPARα, but are also authentic ligands for this receptor [Devchand, P. R. *et al., Nature 384:* 39-43 (1996)]. PPARα is expressed mainly in tissues that have a high level of fatty acid catabolism such as the liver [Braissant, O. *et al., Endocrinology 137:* 354-366 (1995)]. In the liver, PPARα is responsible for the oxidation of fatty acids and the detoxification of several xenobiotic compounds.

Numerous studies have demonstrated that several genes involved in these metabolic pathways, such as the β- and ω-oxidation pathways, contain a PPRE in their promoter regions and are under transcriptional control of PPARα [Reviews: Schoonjans, K. *et al., Biochim. Biophys. Acta. 1302:* 93-109 (1996); Schoonjans, K. *et al., J. Lipid Res. 37:* 907-925 (1996)]. Consistent with this observation, genes involved in β- and ω-oxidation in apparently healthy PPARα knockout mice are not induced when the mice are treated with compounds that activate PPARα [Lee, S. S. T. *et al., Mol. Cell. Biol. 15:* 3012-3022 (1995)].

Fatty acid oxidation pathways play various roles in human physiology, ranging from a role in lipid metabolism to a role in the metabolism of various lipid mediators and signaling factors. Several classes of eicosanoids, including prostaglandins and leukotrienes, have potent pro- or anti-inflammatory actions. These eicosanoids are derived from arachidonic acid, a membrane derived fatty acid, by the action of cyclo- and lipo-oxygenases. It has been known for some time that these eicosanoids are activators of PPARα, and they have now also been shown to be direct ligands of this PPAR subtype [Devchand, P. R. *et al., Nature 384*: 39-43 (1996)].

It has also been shown that PPARα expression can be induced by transcriptional mechanisms upon treatment with anti-inflammatory glucocorticoids [Lemberger, T. *et al., J. Biol. Chem. 269*: 24527-24530 (1994)], an effect that is attenuated by insulin [Steineger, H. H. *et al., Eur. J. Biochem. 225:* 967-974 (1994)].

II. PPARγ

PPARγ was the first PPAR for which ligands were identified. In rodents, PPARγ was thought to be confined to adipose tissue. However, low levels of PPARγ expression were detected in other tissues. This led to the suggestion that PPARγ is a key factor triggering adipocyte differentiation, a hypothesis later confirmed [Spiegelman, B. M. and Flier, J. S., *Cell 87:* 377-389 (1996)]. At least two forms of human PPARγ, γ1 and γ2, have been identified; PPARγ1 has been shown to be the most common form in humans [Mukherjee, R. *et al., J. Biol. Chem. 272:* 8071-8076 (1997)].

PPREs have been identified in several genes that play crucial roles in adipocyte differentiation, most of them affecting lipid storage and control of metabolism. Examples are fatty acid binding protein (aP2) [Tontonoz, P. *et al., Genes Dev. 8:* 1224-1234 (1994)], phosphoenolpyruvate carboxykinase (PEPCK) [Tontonoz, P. *et al., Mol. Cell. Biol. 15:* 351-357 (1995)], Acyl CoA Synthase (ACS) [Schoonjans, K. *et al., Eur. J. Biochem. 216:* 615-622 (1993); Schoonjans, K. *et al., J. Biol. Chem. 270:* 19269-19276 (1995)], and lipoprotein lipase (LPL) [Schoonjans, K. *et al., EMBO J. 15:* 5336-5348 (1996)], all of which are regulated by PPARγ.

Recently, prostaglandin J2 (PGJ2) was shown to be a naturally occurring ligand [Forman, B. M. *et al., Cell 83:* 803-812 (1995); Kliewer, S. A. *et al., Cell 83:* 813-819 (1995)] and the anti-diabetic thiazolidinediones (TZDs) [Forman, B. M. *et al., Cell 83:* 803-812 (1995); Lehmann, J. M. *et al., J. Biol. Chem. 270:* 12953-12956 (1995)] were shown to be synthetic ligands for PPARγ. The identification of PGJ2 and TZDs as PPARγ ligands corroborates the earlier observation that both prostanoids and TZDs are potent inducers of adipose differentiation programs [Gaillard, D. *et al., Biochem. J. 257:* 389-397 (1989); Negrel, R. *et al., Biochem. J. 257:* 399-405 (1989); Forman, B. M. *et al., Cell 83:* 803-812 (1995); Kliewer, S. A. *et al., Cell 83:* 813-819 (1995); Aubert, J. *et al., FEBS Lett. 397:* 117-121 (1996)]. TZDs are currently being developed as insulin sensitizers for the treatment of non-insulin dependent diabetes mellitus (NIDDM)

[Reviews: Hulin, B. *et al., Current Pharm. Design 2:* 85-102 (1996); Saltiel, A. R. and Olefsky, J. M., *Diabetes 45:* 1661-1669 (1996)]. Interestingly, their relative potency to activate PPARγ *in vitro* correlates well with their anti-diabetic potency *in vivo*, suggesting that PPARγ mediates their anti-diabetic effect [Berger, J. *et al., Endocrinology 137:* 4189-4195 (1996); Willson, T. M. *et al., J. Med. Chem. 39:* 665-668 (1996)]. These observations define the role of PPARγ in adipose differentiation and a role in glucose and lipid metabolism.

B. Gastrointestinal Disease

Gastrointestinal disorders are very frequent in Western populations. The most prevalent of these disorders are: peptic ulcer disease, inflammatory bowel disease such as Crohn's disease and colitis ulcerosa, and intestinal neoplasias, such as gastric and colon cancer.

"Peptic ulcer disease" is used to refer to a group of ulcerative disorders of the upper GI tract that appear to have acid-pepsin formation in common in their pathogenesis. The major forms of peptic ulcer are duodenal and gastric ulcer and the Zollinger-Ellison syndrome. The presence or absence of peptic ulcer is determined by the delicate interplay between gastric acid secretion and mucosal resistance. Peptic ulcer disease is present when the aggressive effects of acid-pepsin dominate over the protective effects of gastric or duodenal mucosa. Recently it was shown that colonization with *Helicobacter pylori* is one of the factors that predisposes the development of peptic ulcer disease. Peptic ulcer is common; approximately 10 % of the population have clinical evidence of peptic ulcer during their lifetimes. The diagnosis should be suspected when a patient complains of epigastric pain that is relieved by food intake. Diagnosis is confirmed by radiologic and endoscopic examination, combined with histological analysis of an endoscopic biopsy specimen. Current treatment options include diet, antacids, H2 receptor antagonists, anticholinergic agents, bismuth, omeprazole, and sucrafate. Most recently, antibiotic therapy designed to eliminate *Helicobacter pylori* has been shown helpful in the medical management of peptic ulcer disease. Despite the array of therapeutic agents available for use against peptic ulcer disease, recurrence rates are high, suggesting that there is still room for improvement of medical therapy.

"Inflammatory bowel disease (IBD)" is a general term that encompasses chronic inflammatory disorders of unknown origin involving the GI tract. Chronic IBD may be divided in two major groups: ulcerative colitis and Crohn's disease. While the causes of IBD remain unknown, it is certain that genetic, infectious, immunologic and environmental factors contribute to the disease. IBD is more common in Western populations, and the frequency of its diagnosis has been increasing. In Europe the disease shows a North-South gradient with its highest incidence in the North. Colitis ulcerosa and Crohn's disease have an estimated prevalence of 115/100,000 and 30/100,000, respectively. The complaints and physical findings are specific and consist of abdominal pain and distress, fever, malaise, malabsorption, weight loss, and (bloody) diarrhea. Diagnosis relies on radiologic and endoscopic examination, combined with histological analysis of an endoscopic biopsy specimen. IBD is a severe disorder that shows a chronic and intermittent course and is still associated with a significant mortality (approximately 5%). Besides supportive medical treatment, the medical treatment consists of sulfasalazine and antibiotics (metronidazole, ciproflaxin) and is sometimes accompanied by immunosuppressive therapy (corticosteroids or drugs as azathioprine, methotrexate or cyclosporin). Surgical treatment is usually reserved for disease resistant to medical approach or for complications of IBD, such as intestinal obstruction or perforation. There is currently a large unmet need for more effective and specific medical treatment options for IBD, since most current therapeutic modalities are associated with significant morbidity and have important, potentially serious side effects.

Cancers of the gastrointestinal tract are also frequently diagnosed. Gastric cancer is frequently seen in Japan and in parts of South America and Eastern Europe, whereas it is less frequent in the United States. Colon cancer, on the other hand, is frequent in the United States where it accounts for 20 % of all cancer related deaths. Symptoms of these cancers are nonspecific. Anorexia, weight loss, blood in the stool, change in bowel habits, and fatigue are often-heard complaints. Diagnosis relies on radiological and endoscopic examination and anatomopathological analysis of a biopsy specimen, and is complemented by a careful staging, evaluating the presence of potential metastasis. Curative therapy consists of surgical resection of the affected part of the GI tract. Chemo- and radiotherapy can be combined with surgical intervention especially in the case of metastatic cancers. The 5-year survival rate for a curative surgical intervention is 50 % for

both colon and gastric cancer. Nevertheless prognosis for gastric cancer is worse because it is often diagnosed at a later stage and has a more aggressive nature.

Summary of the Invention

Within the scope of this invention, applicant has discovered that Peroxisome Proliferator Activated Receptors (PPARs) are present in the human gastrointestinal (GI) tract and a variety of GI diseases may be treated with modulators of PPARs, particularly modulators of PPARγ.

Accordingly, the present invention relates to methods and compositions for treating a host having a gastrointestinal (GI) disease by administering to the host a composition containing a pharmaceutically effective amount of a modulator of a Peroxisome Proliferator Activated Receptor (PPAR), particularly a modulator of PPARγ. The host may be a human patient or an animal model of human GI disease. The compositions of this invention are adapted to cure, improve or prevent one or more symptoms of GI disease in the host. A preferred composition is highly potent and selective with low toxicity.

By pharmaceutically effective amount is meant an amount of a pharmaceutical compound or composition having a therapeutically relevant effect on a GI disease. A therapeutically relevant effect relieves to some extent one or more symptoms of GI disease in a patient or returns to normal either partially or completely one or more physiological or biochemical parameters associated with or causative of GI disease, including, but not limited to, malaise, fever, abdominal pain and distress, malabsorption, diarrhea, blood loss through stool or vomit, weight loss, anemia, leukocytosis, abnormal endoscopic or radiologic appearance of GI tract.

GI diseases that may be treated by the methods and compositions of the present invention are diseases that adversely affect the GI tract, in particular, inflammatory, proliferatory and ulcerative diseases of the GI tract, including, but not limited to, peptic ulcer disease, colon cancer, and inflammatory bowel disorders such as colitis ulcerosa and Crohn's disease. Among the GI diseases that may be treated by the present invention are GI diseases that are caused by, or result in, altered PPAR activity in the GI tract. Such GI diseases may be treated by administration of a PPAR modulator that restores PPAR activity in the GI tract partially or completely to normal levels present before the onset of the GI disease. Such GI diseases that may be treated with a PPAR modulator according to

this aspect of the invention may be identified based on the effect of the disease upon PPAR activity in the GI tract. GI diseases with reduced levels of PPAR activity in the GI tract are treated with modulators that activate PPARs, such as PPAR agonists or agents that enhance PPAR gene expression. GI diseases with increased levels of PPAR activity in the GI tract are treated with modulators that inhibit PPARs, such as PPAR antagonists or agents that inhibit PPAR gene expression.

PPAR modulators useful in treating GI diseases according to the invention are small molecules and non-naturally occurring fatty acids that modulate the activity of one or more PPARs. Such PPAR modulators include PPAR agonists and antagonists and agents that alter PPAR expression levels.

By PPAR agonist is meant a compound or composition that, when combined with a PPAR, increases a reaction typical for the receptor, e.g., transcriptional regulation activity, as measured by an assay known to one skilled in the art, including, but not limited to, the co-transfection or cis-trans assays described or disclosed in U.S. Patent Nos. 4,981,784 and 5,071,773 and Lehmann, J. M. *et al., J. Biol. Chem. 270*: 12953-12956 (1995), which are incorporated by reference herein. A preferred PPARγ agonist is a thiazolidinedione compound, including, but not limited to, BRL 49653, troglitazone, pioglitazone, ciglitazone, WAY-120,744, englitazone, AD 5075, darglitazone, 15-Deoxy-D12,14 prostaglandin J2, and congeners, analogs, derivatives and pharmaceutically acceptable salts thereof. Compounds disclosed in Forman, B. M. *et al., Cell. 83*: 803-812 (1995); Tontonez, P. *et al., Genes & Develop. 8*: 1224-1234 (1994); Tontonez, P. *et al., Cell 79*: 1147-1156 (1994); Lehmann, J. M. *et al., J. Biol. Chem. 270(22):* 1-4 (1995); Amri, E.-Z. *et al., J. Lipid Res. 32*: 1449-1456 (1991); Amri, E.-Z. *et al., J. Lipid Res. 32*: 1457-1463 (1991) and Grimaldi *et al., Proc. Natl. Acad. Sci. USA 89*: 10930-10934 (1992) are incorporated by reference herein. A preferred PPARα agonist is a fibrate compound including, but not limited to, gemfibrozil, fenofibrate, bezofibrate, clofibrate, ciprofibrate, and analogs, derivatives and pharmaceutically acceptable salts thereof. Preferred PPARα and γ agonists include sulfur-substituted fatty acids and derivatives hereof, 5,8,11,14-eicosatetraynoic acid (ETYA). Preferred PPARβ agonists include sulfur-substituted fatty acids and derivatives hereof, ETYA and compounds disclosed in U.S. Patent No. 5,093,365.

In a preferred embodiment, an RXR ligand is used in place of or in addition to the PPAR agonist in the composition or method of this invention. By RXR ligand is meant a compound or composition that, when combined with RXR, increases the transcriptional regulation activity of the RXR/PPAR heterodimer, as measured by an assay known to one skilled in the art, including, but not limited to, the co-transfection or cis-trans assays described or disclosed in U.S. Patent Nos. 4,981,784; 5,071,773; 5,298,429; 5,506,102; WO89/05355; WO91/06677; WO92/05447; WO93/11235; WO95/18380; PCT/US93/04399; PCT/US94/03795; and CA 2,034,220, which are incorporated by reference herein. It includes RXR agonists that, when combined with RXR homodimers or heterodimers, increase the transcriptional regulation activity of both the RXR homodimers and heterodimers. It also includes RXR antagonists that increase the transcriptional regulation activity of RXR/PPAR heterodimers while decreasing the transcriptional regulation activity of RXR homodimers, including, but not limited to, LG100754, *i.e.* (2E,4E,6Z)-3-methyl-7-[3-*n*-propoxy-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalen-2-yl)-octa-2,4,6-trienoic acid, LG100823, *i.e.* (2E,4E,6Z)-7-(3-benzyloxy-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalen-2-yl)-3-methylocta-2,4,6-trienoic acid, LG100541, *i.e.* 4-(3,4,5,6,7,8-hexahydro-5,5,8,8-tetramethylanthracen-1-ylmethyl)-benzoic acid, and cogeners, analogs, derivatives and pharmaceutically acceptable salts thereof. It includes, but is not limited to, compounds that preferentially activate RXR over RAR (i.e. RXR specific agonists), and compounds that activate both RXR and RAR (i.e. pan agonists). It also includes compounds that activate RXR in a certain cellular context but not in others (i.e. partial agonists). Compounds disclosed or described in the following articles, patents and patent applications that have RXR agonist activity are incorporated by reference herein: U.S. patents 5,399,586 and 5,466,861; WO96/05165; PCT/US95/16842; PCT/US95/16695; PCT/US93/10094; WO94/15901; PCT/US92/11214; WO93/11755; PCT/US93/10166; PCT/US93/10204; WO94/15902; PCT/US93/03944; WO93/21146; provisional applications 60,004,897 and 60,009,884; Boehm, M. F. *et al., J. Med. Chem. 38(16):* 3146-3155 (1994); Boehm, M. F. *et al., J. Med. Chem. 37(18):* 2930-2941 (1994); Antras *et al., J. Biol. Chem. 266:* 1157-1161 (1991); Salazar-Olivo *et al., Biochem. Biophys. Res. Commun. 204:* 157-263 (1994); and Safanova, *Mol. Cell. Endocrin. 104:* 201-211 (1994). RXR specific ligands include, but are not limited to, LG100268 (i.e. 2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-

naphthyl)-cyclopropyl]-pyridine-5-carboxylic acid) and LGD 1069 (i.e. 4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-2-carbonyl]-benzoic acid), and congeners, analogs, derivatives and pharmaceutically acceptable salts thereof. The structures and syntheses of LG100268 and LGD 1069 are disclosed in Boehm, M. F. *et al., J. Med. Chem. 38(16):* 3146-3155 (1994), incorporated by reference herein. Pan agonists include, but are not limited to, ALRT 1057 (9-*cis* retinoic acid), and analogs, derivatives and pharmaceutically acceptable salts thereof.

By PPAR antagonist is meant a compound or composition that, when combined with a PPAR, decreases a reaction typical for the receptor, e.g., transcriptional regulation activity, as measured by an assay known to one skilled in the art, including, but not limited to, the co-transfection or cis-trans assays described or disclosed in U.S. Patent Nos. 4,981,784 and 5,071,773 and Lehmann, J. M. *et al., J. Biol. Chem. 270:* 12953-12956 (1995), which are incorporated by reference herein. PPAR antagonists include any compound (*e.g.,* an anti-PPAR antibody, etc.) that prevents or inhibits the association of a functional heterodimer by binding to one or both of the heterodimer partners.

A composition containing a pharmaceutically effective amount of a PPAR modulator according to the invention may be administered orally or systemically to a host. In a preferred embodiment, it is administered orally or via the rectum.

The skilled artisan can determine the appropriate PPAR modulator(s) to use in any particular circumstance based upon the GI disease to be treated, the symptoms exhibited by the host to be treated, the level of PPAR activity in the GI tract of the host, and other factors well known to those of skill in the art.

This invention features a pharmaceutical composition for treating GI disease containing a pharmaceutically effective amount of a PPAR modulator and a pharmaceutically acceptable carrier adapted for a host, particularly a human, having a GI disease.

In a preferred embodiment, the composition is held within a container that includes a label stating to the effect that the composition is approved by the FDA in the United States (or an equivalent regulatory agency in a foreign country) for treating GI disease. Such a container provides a therapeutically effective amount of the active ingredient to be administered to a host.

This invention also features screening methods for identifying PPAR modulators that are useful for treating GI disease. These screening methods utilize cells derived from gastrointestinal tissue which express PPARs.

Other features and advantages of the invention will be apparent from the detailed description of the invention below and from the list of enumerated embodiments that follows.

Brief Description of the Drawing

Figure 1 shows the expression levels of PPARγ1 and PPARγ2 in different human tissues.

Detailed Description of the Invention

Pharmaceutical Formulations and Modes of Administration

The particular compound(s) that affects the disorders or conditions of interest can be administered to a patient either by itself, or in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s). In treating a patient exhibiting a disorder of interest, a therapeutically effective amount of an agent or agents such as these is administered. A therapeutically effective dose refers to that amount of the compound that results in amelioration of symptoms or a prolongation of survival in a patient.

The compounds also can be prepared as pharmaceutically acceptable salts. Examples of pharmaceutically acceptable salts include acid addition salts such as those containing hydrochloride, sulfate, phosphate, sulfamate, acetate, citrate, lactate, tartrate, methanesulfonate, ethanesulfonate, benzenesulfonate, *p*-toluenesulfonate, cyclohexylsulfamate and quinate. (See, *e.g.*, PCT/US92/03736.) Such salts can be derived using acids such as hydrochloric acid, sulfuric acid, phosphoric acid, sulfamic acid, acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, *p*-toluenesulfonic acid, cyclohexylsulfamic acid, and quinic acid.

Pharmaceutically acceptable salts can be prepared by standard techniques. For example, the free base form of the compound is first dissolved in a suitable solvent such as an aqueous or aqueous-alcohol solution, containing the appropriate acid. The salt is then

isolated by evaporating the solution. In another example, the salt is prepared by reacting the free base and acid in an organic solvent.

Carriers or excipients can be used to facilitate administration of the compound, for example, to increase the solubility of the compound. Examples of carriers and excipients include calcium carbonate, calcium phosphate, various sugars or types of starch, cellulose derivatives, gelatin, vegetable oils, polyethylene glycols and physiologically compatible solvents.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, for example, for determining the LD^{50} (the dose lethal to 50% of the population) and the ED^{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects, the therapeutic index, can be expressed as the ratio LD^{50}/ED^{50}. Compounds that exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosages for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the $ED^{50}{}_5$with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. Levels in plasma may be measured, for example, by HPLC.

The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. [See, *e.g.*, Fingl *et al.*, in *The Pharmacological Basis of Therapeutics Ch. 1:* 1 (1975)]. It should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity, or to organ dysfunction. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administrated dose in the management of the disorder of interest will vary with the severity of the condition to be treated and to the route of administration. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency, will also vary according to the age, body weight, and response of the individual patient. A program comparable to that discussed above may be used in veterinary medicine.

Depending on the specific conditions being treated, such agents may be formulated and administered systemically or locally. Techniques for formulation and administration may be found in *Remington's Pharmaceutical Sciences*, 18th ed., Mack Publishing Co., Easton, PA (1990). Suitable routes may include oral, rectal, transdermal, vaginal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections.

For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For such transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

Use of pharmaceutically acceptable carriers to formulate the compounds herein disclosed for the practice of the invention into dosages suitable for systemic administration is within the scope of the invention. With proper choice of carrier and suitable manufacturing practice, the compositions of the present invention, in particular, those formulated as solutions, may be administered parenterally, such as by intravenous injection. The compounds can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the invention to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated.

Agents intended to be administered intracellularly may be administered using techniques well known to those of ordinary skill in the art. For example, such agents may be encapsulated into liposomes, then administered as described above. Liposomes are spherical lipid bilayers with aqueous interiors. All molecules present in an aqueous solution at the time of liposome formation are incorporated into the aqueous interior. The liposomal contents are both protected from the external microenvironment and, because liposomes fuse with cell membranes, are efficiently delivered into the cell cytoplasm. Additionally, due to their hydrophobicity, small organic molecules may be directly administered intracellularly.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries that facilitate processing of the active compounds into preparations that can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions. The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, for example, by means of conventional mixing, dissolving, granulating, dragee-making, levitating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. Liposomes may be used for encapsulated delivery.

Agents intended to be administered via the rectum may be administered using techniques well known to those of ordinary skill in the art.

Pharmaceutical formulations disclosed or described in Boehm, *et al.*, WO94/15902 are incorporated by reference herein.

All publications referenced are incorporated by reference herein, including the nucleic acid sequences and amino acid sequences listed in each publication. All the compounds disclosed and referred to in the publications mentioned above are incorporated by reference herein, including those compounds disclosed and referred to in articles cited by the publications mentioned above.

Screening Methods

In another aspect of the present invention, cells derived from gastrointestinal tissue are used to screen for PPAR modulators using a conventional approach that has been used with cells from non-gastrointestinal tissue to screen for mimics and modulators of several cellular signals (*see, e.g.* Rosen *et al., J Med. Chem 38*: 4855 (1995); Lehmann, *et al., J. Biol. Chem. 270*:12953-12956 (1995); U.S. Patent Nos. 4,981,784, 5,071,773, 5,298,429, 5,506,102, WO89/05355, WO91/06677, WO92/05447, WO93/11235, WO95/18380,

PCT/US93/04399, PCT/US94/03795 and CA 2,034,220, which are incorporated by reference herein).

According to this aspect of the invention, cells derived from gastrointestinal tissue which express one or more PPARs may be used to screen for PPAR modulators. These include, but are not limited to, Caco-2 (ATCC No. HTB-37) and C2BBe1 (ATCC CRL-2102) cells derived from human colon adenocarinoma tissue [Fogh, J., *J. Natl. Cancer Institute (Bethesda) 58:* 209-214 (1977); Fogh, J., *J. Natl. Cancer Institute (Bethesda) 59:* 221-226 (1977); Petersen, M.D. *et al., J. Cell Sci. 102:* 581-600 (1992)], and IEC-6 cells derived from rat small intestine (Quaroni, A., *J. Cell Biol. 80:* 248-265 (1979).

In this approach, the ability of a compound to act as a PPAR modulator is measured by the ability of the compound to alter the expression of one or more target genes whose expression is mediated by PPAR in the host cell used. PPAR responsive target genes which may be used in the assay include genes which naturally occur in the host cell used, as well as genes which are engineered for PPAR responsiveness and transfected into the host cell. In a preferred embodiment, the target gene used is a gene encoding luciferase which has been engineered for PPAR responsiveness.

Using this assay, compounds may be assayed for their ability to activate PPAR or to alter the activity of an activated PPAR. To assay for activation of PPAR, the level of expression of one or more PPAR-responsive target genes is measured both in the presence and absence of a test compound and the measurements are compared. PPAR activators are identified as those compounds which alter the expression of a target gene in the same manner, but not necessarily to the same degree, as a known PPAR activator such as BRL 49653 or troglitazone.

To detect modulators of a PPAR activator, the level of expression of one or more PPAR-responsive target genes is measured (1) in the presence of both an activator and a test compound, and (2) in the presence of the activator alone, and the measurements are compared. Those compounds which alter the response of the target gene are identified as modulators of the PPAR activator. Those compounds which increase the level of expression of a target gene in response to a PPAR activator are identified as potentiators of the activator and those which decrease the level of expression of a target gene in response to a PPAR activator are identified as inhibitors or antagonists of the activator.

Expression of a target gene can be measured at the level of transcription by measuring mRNA levels, or at the level of translation by measuring protein levels or protein activity. Techniques which are well known in the art for measuring mRNA or protein levels can be used in the methods of the invention to measure the level of expression of a target gene. Standard techniques for detecting and quantitating mRNA levels include, but are not limited to, Northern blot analysis, S1 nuclease analysis, RNase protection and PCR. Standard techniques for detecting and quantitating protein levels include, but are not limited to, Western blot analysis, ELISA, and activity assays for particular proteins such as enzymes and reporters, e.g. luciferase [E. Harlow *et al.*, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1988) De-Wet *et al., Mol. Cell. Biol. 7*: 725 (1987)].

Compounds identified by the methods of the invention may be used as drugs for modulating PPAR activity in the gastrointestinal tract as described herein.

Example 1:Colorectal Cancer and PPARs

Colorectal cancer, one of the most frequent cancers in the industrialized world, has been shown to be a paradigm for the multistep genetic basis of cancer [1]. The intake of fatty acids is correlated with susceptibility for colon cancer [7-14]. It is also known that decreased production of arachidonic acid-derived prostaglandins prevents or attenuates colon cancer development as observed in mice with mutations in the inducible cyclooxygenase (COX) 2 gene, and in animals and human treated with specific COX inhibitors such as aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) [2-6]. 15-deoxy-$D^{12,14}$-prostaglandin J_2 has been shown to be a natural ligand for PPARγ [18, 21].

These observations led applicant to study downstream transcriptional mediators for prostaglandins and fatty acids, such as the peroxisome proliferator-activated receptor g (PPARγ) (reviewed in [15])

PPARγ is a nuclear receptor implicated in adipocyte differentiation and insulin sensitivity [16-20]. Antidiabetic thiazolidinediones, such as troglitazone and BRL-49,653, are high affinity synthetic ligands for PPARγ and improve insulin sensitivity and glucose homeostasis through the activation of PPARγ [17]. The fact that PPARγ was activated by prostaglandins and fatty acids and the fact that high levels of PPARγ mRNA are detected

in large intestine in animals and humans[22,23] made this receptor a good candidate gene to study for colon carcinogenesis.

Animal model of colorectal cancer

A mouse model for colorectal cancer, C57BL/6J-APC^{Min} / + mice, was used to study the role of PPARγ in colon cancer development. Heterozygous C57BL/6J-APC^{Min} / + mice carry a non-sense mutation at codon 850 in adenomatous polyposis coli (APC) tumor suppressor gene [24,25] and develop ± 30 tumors throughout the intestinal tract associated with a reduced life-span of about 120 days [24]. The human APC gene which plays a similar "gatekeeping" function in colorectal cancer is not only frequently mutated in familial adenomatous polyposis (FAP) [26-29] but also in sporadic colon cancer [29].

Only the colon expresses high levels of PPARγ

The colon is the only part of the intestine showing high levels of PPARγ by Northern and Western blot analysis. Expression of PPARγ in colon is comparable to that in White Adipose tissue. Immunohistochemical staining of PPARγ in sections of normal mouse colon revealed that PPARγ is expressed both in epithelial cells and in the muscular layer, and most abundantly located in the nuclei.

PPARγ activators increase tumor multiplicity and size in the colon

Heterozygote male C57BL/6J-APC^{Min} / + mice in this study developed a mean of ± 25 tumors (26.9 ± 12.9 tumors in the first experiment; 22.0 ± 7.7 in the second experiment) in their gastro-intestinal tract. The highest frequency of tumors is found in the small intestine whereas lower numbers are found in the colon.

Tumor number and size in C57BL/6J-APC^{Min} / + untreated control mice were compared with mice treated with the highly selective PPARγ agonists BRL-49,653 (20 mg/kg/d) or troglitazone (150 mg/kg/d) in an 8 week period.

A 5-fold increase in tumor multiplicity in the colon (2.89±1.4 vs 0.55±0.9) was observed after treatment with BRL-49,653. A 3-fold (1.7±1.0 vs 0.6±0.5) increase was seen with the weaker ligand, troglitazone. Both BRL-49,653 and troglitazone favored the formation of larger tumors as evidenced by the 4-fold increase in tumors with a size

superior to 5 mm. This effect of PPARγ ligands on tumor size completely accounted for the increased numbers of large-sized tumors in the colon.

The strong promotion of colon tumor formation by PPARγ agonists is in striking contrast to the neutral effect of PPARγ agonists on tumor formation in the remainder of the gastrointestinal tract.

Mice treated with PPARγ activators have less differentiated tumors in the colon

Histological analysis of colon lesions in control C57BL/6J-APC^{Min} / + mice revealed that the tumors were mainly well differentiated tubular adenomas. In BRL-49,653 treated mice, however, 57% of the tumors were adenocarcinomas.

The diagnosis of adenocarcinoma was based upon cytological and architectural abnormalities which were much more pronounced than in the adenomas. Cytological abnormalities include cellular and nuclear pleiomorphism and loss of polarity. Architectural abnormalities are the presence of intraglandular papillary projections and of cribriform and solid epithelial areas.

In the adenocarcinomas a cribriform growth pattern was seen in more than 50 % of the tumor area. By immunostaining with a PPARγ specific antibody, the presence of PPARγ was confirmed in adenocarcinomas in the colon of these C57BL/6J-APCmin/+ mice. Similar histological results were observed after troglitazone treatment.

PPARγ activators increase the level of β-catenin in the colon

The effects of PPARγ activation on the expression of some key proteins involved in colon carcinogenesis, such as β-catenin and COX-2, was analysed. Both β-catenin and COX-2 are detected in distal intestine and in colon, but not in the proximal intestine.

Treatment with BRL-49,653 significantly increased the expression of β-catenin, as determined by immunoblotting of protein extracts of the colon of C57BL/6J-APC^{Min} / + mice. Similar results were obtained for troglitazone. These data support that PPARγ could influence the development of colon tumors by changing the concentrations of β-catenin.

Consistent with previous observations, the inducible COX-2 expression was increased in the distal intestine of C57BL/6J-APC^{Min} / + mice relative to the proximal duodenal mucosa. No effect of PPARγ activation, however, was observed on COX-2 protein levels in the colon.

PPARγ activators increases β-catenin expression in HT-29 adenocarcinoma cells

The effects of PPARγ activation on β-catenin expression was also studied *in vitro* in the cultured human colon carcinoma cell line, HT-29. HT-29 cells express high levels of PPARγ protein. Treatment with PPARγ activators did not change the levels of PPARγ in these cells.

The effect of PPARγ activation on the expression of β-catenin and COX-2 was also evaluated. BRL-49,653 treatment induced β-catenin protein levels by 2-fold in HT-29 cells but had no effects on COX-2 protein level, supporting that the effects of PPARγ activation were independent of changes in COX-2 levels.

Treatment of C57BL/6J-APC^{Min} / + mice, an animal model for the study of colorectal cancer, with PPARγ activators significantly increases frequency and size, and decreases differentiation of tumors in the colon, but not in the rest of the intestine. This increase in tumor number in C57BL/6J-APC^{Min} / + mice after treatment with two different synthetic PPARγ activators is consistent with the increase in tumor number observed after a high-fat diet reported by Wasan et al.[12]

It has been known that the increase in tumors after a high fat diet was proportionally higher in the colon as compared with the small intestine. Without being bound by any theory, applicant proposes that alterations in fatty acid concentration or composition, which are induced by dietary changes, influence colon function by changing the activity of PPARγ, a well established fatty-acid-responsive transcription factor.

PPARγ was expressed at a high level in the colon. Much lower levels of PPARγ were present in the rest of the intestine. The correlation between high levels of PPARγ and intestinal regions with increased tumor formation supports that activation of PPARγ in the colon influences tumor initiation and/or development in the C57BL/6J-APC^{Min} / + mouse model.

Our data show an important effect of PPARγ activation by synthetic agonists on the development of colon cancer in the C57BL/6J-APC^{Min} / + mouse, a model which is clinically relevant since it mimics APC gene inactivation, observed both in human familial adenomatous polyposis [26, 27], as well as in sporadic cases of colon cancer [29]. PPARγ may be involved with mediating some of the deleterious effects of Westernized diets, high in animal fat and simple carbohydrates, on colon cancer. Finally, these data caution against long-term use of hypoglycemic agents of the thiazolidinedione group.

Materials and Methods

Reagents and antibodies

Troglitazone and BRL-49,653 were obtained from Dr. A. Saltiel (Parke-Davis) and Dr. A. Nazdan (Ligand Pharmaceuticals, Inc.), respectively. A rabbit IgG anti-human PPARγ was developped against an N-terminal human PPARγ peptide (amino acids 20-104) and used at a 1:500 dilution as previously described [23]. Goat anti-Cox-2 (sc-1746), goat anti-β-catenin (sc-1496) were purchased from Santa-Cruz (Santa-Cruz, USA) and used at a 1:500 dilution.

Animal experiments

All animal experiments were performed according to EU governemental guidelines. Male C57BL/6J-APC^{Min} /+ mice were obtained at 5 weeks of age from the Jackson laboratory (Bar Harbor, Maine, USA) and were group-housed (6-8 per cage) and accustomed to a 12:12 hr day-night illumination cycle (from 8 am to 8 pm). Pure tap water was available *ad libitum* and they received standard mice chow (D04, UAR, France). In two distinct experiments, animals were treated for 8 weeks (starting at 6 weeks of age) with BRL-49,653 (20 mg/kg body weight/day) or troglitazone (150 mg/kg body weight/day) mixed with the standard show. Control mice were fed a diet without drug. New batches of mixture of drug/food were prepared weekly and stored until use at 4°C. A fresh ransom of food was added each day to protected feeders.

Tumor analysis

At the end of the treatment, animals were sacrified by decapitation under light ether anesthesia. The entire intestine was quickly removed, divided in 5 equal sections from duodenum to the rectum, opened and examined under a magnification dissecting microscope (x5) to count the tumors. The tumor diameter was measured with a calibrated eye reticle.

Cell culture

HT-29 cells were grown in McCoy's medium supplemented with 10% fetal calf serum, 50 mg/ml streptomycin and 50u/ml penicillin. The cells were subcultured when they were 80% confluent by using 0.5 % trypsin and seeded at a density of $5x10^4$ cells/cm^2.

RNA and protein analysis

Isolation of total RNA, Northern blot analysis, protein preparation, and immunoblotting were performed as described [30-32]. Immunodetection with secondary peroxidase conjugated antibody and chemilluminescence was performed according to the manufacturer's protocol (ECL, Amersham, UK).

Histology and immunochemistry

Samples of small intestine and colon tissues were fixed in 4% paraformaldehyde acid (PFA) at 4°C overnight, deshydrated in alcohol and embedded in paraffin. Five mm sections were then deparaffined with xylene and rehydrated by ethanol treatment. For routine histology the sections were stained with haematoxylin and eosin. Immunocytochemistry was performed with the PAP universal DAKO Stain Kit according to the manufacturer's protocol (DAKO, Denmark).

In summary, activation of PPARγ by two different synthetic agonists increased the frequency and size and decreased the differentiation of colon tumors in an animal model susceptible to intestinal neoplasia, i.e., C57BL/6J-APC^{Min} / + mice. Tumor frequency was increased in the colon, whereas no change in frequency of the tumors was observed in the small intestine. This correlates with the colon-restricted expression pattern of PPARγ mRNA and protein.

PPARγ agonists induced β-catenin expression both in the colon of C57BL/6J-APC^{Min} / + mice as well as in HT-29 colon carcinoma cells. Genetic abnormalities in the Wnt/wingless/APC pathway, which increase free β-catenin levels and enhance the transcriptional activity of the β-catenin-Tcf/Lef1 transcription complex, have been shown to frequently underly the development of colon cancer. These data support that PPARγ activation modifies the development of colon cancer.

That the PPARγ expression pattern coincides with the regions where PPARγ activation may be associated with cancer development strongly supports that PPARγ is involved in the enhancement of colon tumorigenesis in C57BL/6J-APC^{Min} / + mice.

Example 2:Inflammatory Diseases of the GI Tract and PPARs

Inflammatory cells such as blood monocytes express very low levels of PPARγ in the resting or inactive state (*See, e.g.,* Ricote, et al., Nature 391:79-82 (1998)). The level of expression of PPARγ rises significantly when monocytes are provoked to produce an

inflammatory response as when stimulated with interferon γ. The administration of a PPARγ modulator at this time, such as 15-deocy-D12,14 prostaglandin J2, suppresses inflammatory responses. A PPARγ modulator can suppress the expression of inducible nitric oxide synthetase, the expression of gelatinase B, and the production of inflammatory cytokines such as tumor necrosis factor α (TNF-α) by human monocytes (*See, e.g.,* Jiang, et al., Nature 391:82-86 (1998)). Given the expression of PPARγ in the GI tract, as discovered by the applicant, PPARγ modulators can be used to treat the inflammatory diseases of the GI tract.

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All publications cited in the specification are incorporated by reference herein, including drawings and sequences listed in each publication. All the compounds disclosed and referred to in the publications mentioned above are incorporated by reference herein, including those compounds disclosed and referred to in articles cited by the publications mentioned above.

Other embodiments of this invention are disclosed in the following claims.

We claim:

1. A method for treating a host suffering from a gastrointestinal disease comprising administering a pharmaceutically effective amount of an agent comprising a modulator of a PPAR to said host.

2. The method of claim 1 wherein said host is a human.

3. The method of claim 1 wherein said gastrointestinal disease is selected from the group consisting of an inflammatory disease of the gastrointestinal tract, a proliferatory disease of the gastrointestinal tract, and an ulcerative disease of the gastrointestinal tract.

4. The method of claim 3 wherein said gastrointestinal disease is an inflammatory disease of the gastrointestinal tract.

5. The method of claim 4 wherein said inflammatory disease is selected from the group consisting of colitis ulcerosa, Crohn's disease, and gastritis.

6. The method of claim 3 wherein said gastrointestinal disease is a proliferatory disease of the gastrointestinal tract.

7. The method of claim 6 wherein said proliferatory disease is selected from the group consisting of benign tumors of the gastrointestinal tract, polyps of the gastrointestinal tract, hereditary polyposis syndrome, colon cancer and gastric cancer.

8. The method of claim 7 wherein said proliferatory disease is colon cancer and said modulator is an inhibitor of said PPAR.

9. The method of claim 3 wherein said gastrointestinal disease is an ulcerative disease of the gastrointestinal tract.

10. The method of claim 9 wherein said ulcerative disease is selected from the group consisting of duodenal and gastric ulcer, esophageal ulcer, stress ulcers and erosions, drug-associated erosions, and Zollinger-Ellison Syndrome.

11. The method of claim 1 wherein said PPAR is selected from the group consisting of PPARα, PPARδ and PPARγ.

12. The method of claim 11 wherein said PPAR is PPARγ.

13. The method of claim 1 wherein said agent comprises a combination of two or more PPAR modulators.

14. A method for treating a host suffering from a gastrointestinal disease resulting in an altered level of intestinal activity of a PPAR comprising administering a pharmaceutically effective amount of a modulator of said PPAR to said host, wherein said modulator restores the intestinal activity of said PPAR to the level occurring before the onset of said gastrointestinal disease in said host.

15. A pharmaceutical composition adapted for the treatment of a host having a gastrointestinal disease comprising

(a) a pharmaceutically effective amount of a PPAR modulator; and

(b) a pharmaceutically acceptable carrier.

16. The pharmaceutical composition of claim 15, wherein said pharmaceutical composition is specifically made for rectal administration.

17. A method for measuring the ability of a compound to activate a PPAR, comprising determining the level of expression of a gene responsive to said PPAR in a host cell which expresses said PPAR and is derived from gastrointestinal tissue under conditions in which said gene is expressed in response to said PPAR in an activated state:

(a) in the absence of said compound;

(b) in the presence of said compound; and

comparing the level of expression of said gene from (a) with the level of expression of said gene from (b), wherein the ability of the compound to activate said PPAR is measured as the increase in the level of gene expression in step (b) compared to step (a).

18. The method of claim 17 wherein said host cell is derived from human gastrointestinal tissue.

19. The method of claim 18 wherein said host cell is selected from the group consisting of Caco-2 (ATCC HTB-37) cells and C2BBe1 (ATCC CRL-2102) cells.

20. The method of claim 17 wherein said host cell is derived from rat gastrointestinal tissue.

21. The method of claim 20 wherein said host cell is IEC-6 (ATCC CRL-1592) cells.

22. A method for measuring the ability of a compound to act as a modulator of a PPAR comprising determining the level of expression of a gene responsive to said PPAR in a host cell which expresses said PPAR and is derived from gastrointestinal tissue under conditions in which said gene is expressed in response to said PPAR and a PPAR activator:

(a) in the presence of both said PPAR activator and said compound;

(b) in the presence of said PPAR activator only; and

comparing the level of expression of said gene from (a) with the level of expression of said gene from (b), wherein the ability of said compound to act as a modulator of said PPAR is measured as the amount of difference in the level of gene expression in step (a) compared to step (b).

23. The method of claim 22 wherein said host cell is derived from human gastrointestinal tissue.

24. The method of claim 23 wherein said host cell is selected from the group consisting of Caco-2 (ATCC HTB-37) cells and C2BBe1 (ATCC CRL-2102) cells.

25. The method of claim 22 wherein said host cell is derived from rat gastrointestinal tissue.

26. The method of claim 25 wherein said host cell is IEC-6 (ATCC CRL-1592) cells.

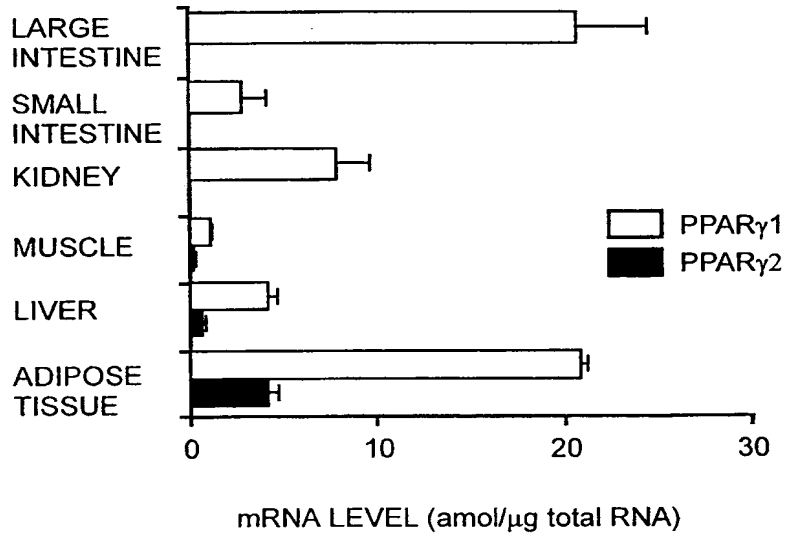
LARGE INTESTINE
SMALL INTESTINE
KIDNEY
MUSCLE
LIVER
ADIPOSE TISSUE
PPARγ1
PPARγ2
0
10
20
30
mRNA LEVEL (amol/μg total RNA)

Fig. 1

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 98/05852

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/50 A61K31/425

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	L.M. BERNSTEIN ET AL.: "Effects of misclerone (clofibrate) on dimethylhydrazine-induced intestinal cacinogenesis in rats" ONCOLOGY, vol. 39, no. 5, 1982, pages 331-335, XP002069292 see the whole document ---	1,3,6-8
A	S.L. PEARSON ET AL.: "The thiazolidinedione insulin sensitiser, BRL 49653, increases the expression of PPAR-gamma and aP2 in adipose tissue of high-fat-fed rats." BIOCHEM. BIOPHYS. RES. COMMUN., vol. 229, no. 3, 1996, pages 752-757, XP002052407 --- -/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 June 1998	09.07.98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Klaver, T

2

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 98/05852

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GB 2 269 897 A (MERCK FROSST CANADA) 23 February 1994	17-26

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/05852

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-14 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No
PCT/US 98/05852

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2269897 A	23-02-1994	NONE	